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Upper Darby, Pa.

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## RESEARCH

### White-Pine Cone Beetle Study

The general life history study of this cone beetle, previously reported in Quarterly Reports 1/, in 1960 and 1961 was supplemented by an investigation of the microenvironment of white pine cones on the forest floor, the hibernation site of the insect. In particular, we wished to know the relationship, if any, between ambient temperatures, cone temperatures, and spring emergence (time and rate).

Briefly, the life history of the white-pine cone beetle, Conophthorus coniperda Schwarz, is as follows: On emergence from the cones in which they overwinter, the adults fly to the upper crowns of white pines. The female beetle bores into the young (second year) cones and constructs a feeding and brood gallery along the axis. First year conelets and new shoots may be fed on but rarely, if ever, contain eggs. Mating apparently takes place inside the cones, and the eggs are laid in niches in the gallery wall. The developing larvae feed on the cone and seed tissues. The infested cones fall prematurely, an average of 38 days after attack. At this time approximately 80 percent of the insects are still eggs or first instar larvae. Development to adults is completed during the summer in the brood cones, and the beetles overwinter in these or other cones on the ground. A few infested cones may remain on the trees, but the white-pine cone beetle is very generally an inhabitant of the ground surface for most of its life cycle and develops under the light, moisture, and temperature regimes there.

In the present study, only two primary variables were measured: (1) beetle emergence in the spring, (2) cone temperature. Supplemental data were obtained on air temperature, humidity, and precipitation.

Infested white pine cones were collected at the Massabesic Experimental Forest, Alfred, Maine, in the fall of 1959. These were stored in screened boxes beneath a white pine overstory at Branford, Connecticut. In early March, 1960, 100 cones were brought into the New Haven Laboratory for dissection. An average of one beetle per cone was found at this time. Two hundred cones were then placed on the bottom of each of eight screened cages, in which pine litter and the cones were arranged so as to

1/ Cf. NEFES, FIR, Quarterly Reports for Oct.-Dec., 1958 and Jan.-March, 1959.

simulate surrounding natural conditions. Four cages were placed in an opening exposed to full sunlight from 9 a.m. to 3 p.m. (E.S.T.). The other four cages were placed beneath some white pines with a minimum of direct exposure to solar radiation (sunlight did reach one or another of these cages, however, between 8:30 and 10:00 a.m. and for 30 minutes in the late afternoon). Low, dense vegetation in the open location reduced air movement there.

Hourly observations and counts of beetle emergence were made from 9 a.m. to 5 p.m. (E.S.T.) beginning about April 1. Only beetles on the top screening of the cages were collected and recorded.

Internal cone temperatures were measured by copper-constantan thermocouples and recorded by a Minneapolis-Honeywell 10-point strip-chart recorder. Each thermocouple was inserted into the beetle gallery (through the exit hole made by the brood mother the previous spring or summer) to approximately the center of the cone. Five cones containing thermocouples were placed at each site, three within cages and two on the litter surface outside.

Air temperature and humidity were measured at 24 inches above the ground in a standard Weather Bureau instrument shelter by a Friez Model 549 hygrothermograph. Precipitation data were taken from U. S. Weather Bureau records for New Haven, Connecticut. The weather station was at the New Haven Municipal Airport about 3 miles from the study area.

These raw data are summarized in the technical report of the study on file at the New Haven Laboratory 2/.

Statistical analysis of the beetle emergence counts, using transformed data, showed no significant difference in the total number emerging within the cages of each site (exposure). The data for the cages in each site were pooled, therefore, to obtain a total for each observation time. Percent beetle emergence was calculated on the basis of number present in the cones that day, until approximately 90 percent of the beetles had emerged. As the emergence period progressed, the numbers of beetles remaining in the cones decreased. Emergence data of May 3, 6, and 12 in the exposed site and May 12, 15, 16, 17, and 19 in the shaded site were not used because of low numbers.

Analysis also showed no significant difference in cone temperatures among the cages in each site, nor in the temperatures of the cones inside and outside the cages. However, the cone temperatures used in the regression analysis (to follow) were averages of the three within-cage thermocouple readings only.

As placed in the cones, the thermocouples measured, in effect, the net resultant of radiation, conduction, convection, and evaporation in

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2/ Godwin, P. A. and T. M. ODell. Some cone beetle-temperature relationships in the microenvironment of white pine cones. Office Rpt. (typed), Forest Insect Lab., Northeast. Forest Expt. Sta., New Haven, Conn. 1962.

the heat balance of the cone. The effects of these four modes of heat transfer were not measured separately. Each might affect insect activity directly, interact with one or more of the others, or interact with other physical factors of the cone microenvironment.

In the present system, evaporation or moisture appeared to have a direct effect beyond the heat transfer process. Both high ambient humidity and precipitation depressed emergence noticeably.

Preliminary analyses of correlation between various temperature indices and percent beetle emergence were made by the quadrant sum method. Five indices were tested: (1) maximum daily temperature, (2) average temperature 6 a.m. to 4 p.m., (3) total number of hours above certain "threshold" temperatures, (4) average number of degree-hours above a threshold temperature, and (5) weighted number of hours above a threshold temperature. These quadrant sum data are given in table 1. As indicated, some correlation was shown with all indices except number of hours above 50°F., 6 a.m. to 4 p.m., and the correlation was generally stronger for the shaded site than the exposed site.

Table 1.--Quadrant sum values for beetle emergence-temperature index relationships of C. coniperda.

| Temperature Index | Quadrant Sums 1/ | | |
|---|---|---|---|
| | Exposed site | Shaded site | Combined data |
| Maximum daily temperature | + 7.5 | +17.5** | +22. ** |
| Average temperature 6 am - 4 pm | +12. * | +15. ** | +19. ** |
| No. hrs. above 50°F.--6 am - 4 pm | + 2. | + 3.5 | + 1. |
| No. hrs. above 60°F.--6 am - 4 pm | + 5. | +13. * | +10. |
| No. hrs. above 65°F.--6 am - 4 pm | + 7. | +15. ** | +19. ** |
| Av. degree-hrs. above 45°F. 6 am - 4 pm | +12. * | +13. * | +19. ** |
| Av. degree-hrs. above 55°F. 6 am - 4 pm | +12. * | +15. * | +19. ** |
| Av. degree-hrs. above 60°F. 6 am - 4 pm | +12. * | +11. * | +19. ** |
| Total weighted hours above 46.4°F. 6 am-4 pm | +12. * | +12. * | +22. ** |
| Total weighted hours above 64.4°F. 6 am-4 pm | +12. * | +13.5* | +23.5** |

1/ A quadrant sum of ±11 is needed for significance at the 5 percent level (*) and ±14 for significance at the 1 percent level (**).

The first two indices considered, maximum daily temperature and average temperature between 6 a.m. and 4 p.m., are self-explanatory. In each case, a time factor was evident -- a higher frequency of zero emergence counts were recorded in the early part of the season regardless of the day's temperature level.

The other indices involve hypothetical "threshold" temperatures, each based on the premise that emergence begins only when a certain temperature is reached. Other insect activities may take place below this temperature, but this threshold is the absolute minimum at which emergence can occur. Obviously, it is necessary to consider the insect emergence-threshold temperature relationship in terms of the length of time each day the threshold level is exceeded as well as the amount by which it is exceeded.

The relationship with degree-hours was analyzed further by means of regression. Equations of the form $Y = b_o + b_iX$ were calculated for both the exposed and shaded sites using daily beetle emergence figures (y) and cumulative degrees Fahrenheit above threshold temperatures of 40°, 45°, 50°, 55°, and 60° (x). For these calculations, the emergence percentages were transformed to probits and the daily cumulative temperatures to logarithms. The calculated equations are given below.

| Threshold temperature | Exposed site | Shaded site |
|---|---|---|
| 60° F. | Y = - 4.11 + 4.84X | Y = - 1.88 + 4.92X |
| 55° F. | Y = - 6.19 + 5.34X | Y = - 4.99 + 5.42X |
| 50° F. | Y = - 7.82 + 5.70X | Y = - 8.18 + 6.11X |
| 45° F. | Y = -10.00 + 6.25X | Y = -13.01 + 7.46X |
| 40° F. | Y = -11.89 + 6.70X | Y = -15.28 + 7.85X |

Tests were made of the regression data of the two sites for each threshold temperature, and in all cases the differences in slope were not significant. The paired lines for the 40° F. and 45°F. thresholds also did not differ as to intercept, or position. The two lines of the 50° F., 55°F., and 60° F. thresholds were significantly different in regard to position and could not, therefore, be combined. The combined site data for 45° F. had the smallest error.

As indices for the quadrant sum tests, the selected "threshold" temperatures were plotted as individual daily values along with their corresponding beetle emergence data. For the regression analysis of the relationship between emergence and degree-hours (index 4), the latter were computed and used as cumulative values. This introduces the concept of a threshold of cumulative temperatures superimposed on or developing from a base temperature. The distinction must be made clear. The base temperature is the minimal level below which no activity of the sort being measured (in this case beetle emergence from white pine cones) occurs. This necessarily must be selected arbitrarily at first, or at best only approximated from knowledge

and experience. If the chosen base temperature is higher than the true base for the particular insect activity, said activity will have been influenced by cumulative temperatures not recorded, and the cumulative temperature value for a given percentage emergence will be less than the true. While this is so in both cool (shaded) and warm (exposed) places, the discrepancy will be most marked for the cool site where a larger proportion of the temperature readings may be below the chosen base. On the other hand, if the assumed base temperature is lower than the true base, an excess of temperature values will be cumulated, and the cumulative temperature value for a given percentage emergence will be greater than the true. In this case, the error is greater in the warmer site where a larger proportion of the temperature readings will be above the assumed base. On the basis of reason alone, it would appear that of any number of arbitrarily chosen base temperatures, that temperature at which there is the smallest difference between the emergence-cumulative temperature relationships of two or more sites must be nearest the true base. In the present study, the regression lines for the shaded and exposed sites were most nearly the same for the data based on the 45° F. "threshold."

In each of the equations, the value of x when Y = 0, i.e., the point where the regression line crosses the abscissa, represents the best estimate of the minimal or threshold cumulative temperature for beetle emergence. The actual value in each case depends on (1) the base temperature used, and (2) what the cumulative temperature values actually comprise (1-hour, 2-hour, 6-hour readings, etc.). Using the 45° F. base and cumulated hourly readings from 6 a.m. to 4 p.m., the estimated number of degree-hours at which cone beetle emergence begins is 46.1.

(ODell, Godwin)

Gypsy Moth Virus Serology Study

A series of immunodiffusion tests of the gypsy moth polyhedral virus were conducted this quarter using the Freer technique. This technique is more sensitive than the "ring test" and permits the identification of multiple antigens in a sample unknown.

Test 1.--Designed to give provisional information on the serological difference, if any, between the polyhedral viruses of Japanese and New Haven strain gypsy moths. Results indicate that both the virus particles and the polyhedral proteins of each are serologically distinct. Further tests will be made.

Test 2.--Made to increase the sensitivity and resolution of the technique by incorporating the antiserum into 1.2 percent clarified agar. Results indicate that this refinement was quite successful. A secondary finding from this test was that the virus and polyhedral protein antisera prepared in chickens was superior to that prepared in rabbits.

Test 3.--A double diffusion test to examine the blood of larvae and the surface and internal contents of eggs of individuals that exhibited a large amount of polyhedrosis during rearing. The blood and egg contents gave negative results, but surface washings of the eggs showed a positive reaction.
(Lewis, Rollinson)

Tests of Nosema spp. against Gypsy Moth

Two species of the protozoan Nosema (lymantriae and serbicae) were obtained from Dr. J. Weiser, Prague, Czechoslovakia, through Dr. A. M. Heimpel, Insect Pathology Pioneering Laboratory, A. R. S. When tested against host gypsy moth larvae, no kill was obtained by N. lymantriae and only a single kill by N. serbicae was recorded. Additional passages of these pathogens are in progress. The addition of a Nosema disease to the arsenal of biotic agents acting against the gypsy moth would be highly desirable, since this type of disease is definitely carried through the egg and becomes progressively more effective in succeeding generations of the host insect. (Lewis, Rollinson)

## MISCELLANEOUS

Personnel

D. Gordon Mott and Gilbert Levesque have joined the staff of the Forest Insect Laboratory, New Haven.

Mr. Mott was formerly at the Forest Entomology and Pathology Laboratory, Fredericton, New Brunswick. As a member of the Green River Project scientific team, he was responsible for studies of the effects of the spruce budworm on the spruce-fir forest and for certain aspects of biometric analysis involving mathematical models of the population dynamics of the budworm. At the New Haven Laboratory he will initiate a comprehensive study of the total impact of destructive insects on northeastern forests.

Mr. Levesque graduated from the University of Maine, majoring in entomology, and received graduate training in this field at Cornell University. His particular interest is in biological control, especially in the quantitative analysis of parasite-host and predator-prey interrelations. He will work with Dr. Robert W. Campbell on the population dynamics of the gypsy moth and associated hardwood defoliators.

In February, Gerald LaVoy, Administrative Assistant, transferred to Station headquarters at Upper Darby, where he will receive high level training in administrative management. Charles L. Crowder has been employed as Administrative Assistant and is in charge of the administrative services unit of the New Haven Laboratory. Mr. Crowder was previously in the personnel section of the Hartford (Conn.) Post Office.

## Meetings

Waters participated in a conference on gypsy moth research with A. R. S. and state cooperators in New York City, February 1. And on February 8 he attended a meeting at Amherst, Mass., with R-7 Forest Pest Control personnel and state cooperators on the development of a regionwide forest pest detection survey program. On February 9, a special session was conducted on the red pine scale problem.

On February 14 Waters addressed the General Forestry Seminar at Yale University on the subject of new horizons and goals in forest insect research.

Lewis and Hastings gave talks on Bacillus thuringiensis, a promising microbial insecticide, and new developments in chemical control of insect pests, respectively, at the Tree Wardens and Arborists Conference, University of Massachusetts, Amherst, on March 12.

Mott and Waters attended the annual meeting of the Northeastern Forest Pest Council in Boston, March 13-14. Waters presented a paper on biological approaches to gypsy moth control.

## Publications and Reports

Campbell, R. W. 1962.
Population dynamics of the gypsy moth. Office Rpt. (typed), Forest Insect Lab., Northeast. Forest Expt. Sta., New Haven. 4 pp. [a special summary of progress to date in the Glenville, N. Y., study, covering the period 1958-61 inclusive.]

Keller, J. C., E. C. Paszek, A. R. Hastings, and V. A. Johnson. 1962.
Insecticide tests against gypsy moth larvae. Jour. Econ. Ent. 55: 102-105.